Research Article

Reduction of Ovarian and Oviductal Cancers in Calorie-Restricted Laying Chickens

Donna K. Carver[1], H. John Barnes[2], Kenneth E. Anderson[1], James N. Petitte[1], Regina Whitaker[3], Andrew Berchuck[3], and Gustavo C. Rodriguez[4]

Abstract

Epithelial ovarian cancer (OVAC) remains a highly lethal malignancy. It is a leading cause of cancer deaths among women in the United States causing more deaths than all other gynecologic malignancies combined. The pathogenesis of OVAC is not completely understood, but the process of repeated ovulation is believed to lead to genetic damage in the ovarian epithelium. As part of a prospective trial designed to evaluate OVAC chemopreventive strategies using the chicken model, caloric restriction (55% less energy) was used to inhibit ovulation in groups of hens receiving chemopreventives, thereby minimizing the impact of ovulation on the incidence of reproductive tract cancer. A separate group of chickens was maintained concurrently in the same environment, and managed similarly, except that caloric intake was not restricted. Among birds not receiving chemopreventive agents, we compared caloric versus noncaloric restricted birds to determine the relations between calorie restriction and risk of developing adenocarcinoma of the reproductive tract. Mortality in the calorie-restricted group was almost half that of those on full feed. Calorie-restricted chickens maintained body weights averaging 1.423 kg compared with the full-fed birds at 1.892 kg. Ovulation rate varied with the full-fed group producing 64% more eggs than the calorie-restricted group. Total reproductive cancers occurred in 57 (33.3%) birds for the full-fed group and 26 (10.3%) birds for the calorie-restricted group. On the basis of histopathology, 45 (26.3%) birds in the full-fed group had ovarian adenocarcinoma compared with 16 (6.3%) birds in the calorie-restricted group. Calorie restriction in laying hens resulted in a near five-fold reduction in OVAC. *Cancer Prev Res; 4(4); 562–7. ©2011 AACR.*

Introduction

Epithelial ovarian cancer (OVAC) remains a highly lethal malignancy. It is the fifth leading cause of cancer deaths among women in the United States and causes more deaths than all other gynecologic malignancies combined (1). The pathogenesis of OVAC is not completely understood, but it is believed that the process of ovulation leads to genetic damage in the ovarian epithelium. OVAC risk correlates with the number of ovulatory cycles in a woman's lifetime, whereas factors associated with decreased ovulation, such as increased parity, breast feeding, and oral contraceptive use, have a protective effect (2–4). These observations have

led to the "incessant ovulation" hypothesis which purports that repeated cycles of epithelial disruption and repair may facilitate neoplastic transformation of the ovarian epithelium in susceptible individuals and that the risk of OVAC may be proportional to the number of ovulatory cycles in a woman's lifetime (5). Repeated cycles of rupture and repair of the ovarian epithelium associated with ovulation may predispose the ovarian epithelium to DNA damage, inclusion cyst formation, and dysplastic changes which can lead to neoplastic transformation.

Lack of a valid OVAC animal model has been a major obstacle to OVAC research. To develop effective therapeutic strategies for OVAC in a timely fashion, animal models that closely mimic human OVAC are desperately needed. Human prevention and treatment trials are costly requiring large numbers of subjects and many years to complete. Development of an animal model for OVAC research would represent a significant breakthrough allowing the expedited evaluation of numerous agents. Ideally, this would lead to the rapid identification of a select number of agents with the greatest potential for OVAC prevention and treatment that could be evaluated in human trials.

Among the candidate OVAC animal models, the domestic fowl may have great potential with characteristics that

Authors' Affiliations: Departments of [1]Poultry Science and [2]Population Health and Pathobiology, N.C. State University, Raleigh; [3]Division of Gynecologic Oncology, Duke University Medical Center, Durham, North Carolina; and [4]Division of Gynecologic Oncology, NorthShore University Health System, University of Chicago, Chicago, Illinois

Corresponding Author: Donna K. Carver, Department of Poultry Science, N.C. State University, CB 7608, Raleigh, NC 27695. Phone: 919-515-5526; Fax: 919-515-7070; E-mail: donna_carver@ncsu.edu

make it especially attractive for chemoprevention research. The most relevant feature of the domestic fowl is its high incidence of *spontaneous* OVAC, which ranges from 11% to 35% between 4 and 6 years of life (6–9). This makes the chicken unique, relative to other animals that require either experimental induction or genetic engineering to induce ovarian tumors (10–33). In addition, the chicken has a high ovulatory rate (daily), raising the possibility that chicken and human OVACs have a common pathogenesis related to ovulation-induced genetic damage to ovarian epithelial cells. Importantly, we have recently shown that chicken ovarian adenocarcinomas have genetic or molecular features that are similar to those of human OVAC (34) and that the prevalence of alterations in p53 in chicken tumors correlated with the number of prior ovulatory events, These findings further support the concept that ovulation with repeated cycles of rupture and repair of the ovarian epithelium may increase the number of proliferative events and genetic errors.

As part of a prospective trial designed to evaluate OVAC chemopreventive strategies using the chicken animal model, caloric restriction was used to inhibit ovulation in groups of hens receiving chemopreventives, thereby minimizing the impact of ovulation as a confounding factor on reproductive tract cancer incidence. A separate group of chickens was maintained concurrently in the same environment and managed similarly, except that caloric intake was not restricted. Among birds not receiving chemopreventive agents, we compared caloric versus noncaloric restricted birds to determine the relation between calorie restriction and risk of developing cancer, specifically adenocarcinoma of the reproductive tract.

Methods

Rearing and husbandry

As part of a 2-year chemoprevention trial designed to evaluate candidate pharmacologic OVAC preventive strategies, 2,400 2-year-old leghorn-laying hens were randomized into 6 groups. Two groups of birds received no chemopreventives but were maintained on either a restricted calorie diet ($n = 394$) or a diet formulated for optimal egg production ($n = 393$). Birds were housed in a cage layer system typical of those used in the table egg layer industry. Birds were maintained with 16.5 hours of light and 7.5 hours of dark. Room temperature was maintained at $80°F \pm 5°F$. Water was provided *ad libitum*. Feed was supplied *ad libitum* with the full-fed group receiving an average of 1,300 kcal/lb of feed and the calorie-restricted group receiving 715 kcal/lb of feed. Birds remained on these diets for 2 years. Eggs were collected daily to determine ovulation rates for the 2 groups. To control for the contribution of mortality to the number of eggs produced, data are reported as number of eggs per bird per month. Mortality was monitored for the 2-year period. Birds found dead were examined grossly and categorized as either a cancer suspect or visibly cancer free. At 4 years of age, surviving birds were weighed, euthanized via cervical dis-

location, and evaluated via necropsy and histopathology by an avian pathologist (H.J.B.) for adenocarcinoma involving the reproductive tract.

Necropsy and tissue collection

All birds were evaluated for ovarian and oviductal adenocarcinoma by gross necropsy. Histopathologic examination was performed on reproductive tracts in 97.7% of birds in the full-fed group and 98.8% of the birds in the calorie-restricted group. Ovarian tumors were staged as follows: stage 1 (confined to ovary), stage 2 (ovary and oviduct affected), stage 3 (ovary and/or oviduct with serosal metastasis), and stage 4 (stage 3 with distant metastasis to liver, spleen, lung, or kidney). Ovary and representative samples of oviduct from each bird were placed into 10% buffered neutral formalin for 72 hours, transferred to 70% ethanol, trimmed, processed by conventional paraffin embedding, sectioned at 5 μm, and stained with hematoxylin and eosin for microscopic examination. When present, lesions in other organs also were similarly processed.

Statistics

Continuous data were analyzed using the General Linear Model's T test in SAS. Dichotomous data were analyzed using the Frequency Procedure's Chi Square test in SAS. Outcome differences with a probability of less than 0.05 were considered significantly different.

Results

Birds in the full-fed group experienced greater overall mortality than birds in the calorie-restricted group (Table 1). Birds eating a calorie-restricted diet died at almost half the rate of those on full feed. By ingesting a diet with 55% less energy, the calorie-restricted group maintained body weights averaging 1.423 kg compared with 1.892 kg for the full-fed group (Table 2). This represents a 25% difference in body weight between the 2 groups. Overall egg production varied for the 2 groups with the full-fed group producing 8.67 eggs per bird per month at peak production compared with 4.47 eggs per bird per month peak production for the calorie-restricted group (Fig. 1). The full-fed group ovulated over 32,000 times during the 2-year period compared with 11,500 for the calorie-restricted group. Gross pathology revealed that

Table 1. Mortality

	Full-fed	Calorie-restricted
Mortality number	261a	143b
Percent mortality,[a] %	62.1	36.3

NOTE: Outcomes with different letters (a and b) were significantly different with $P \leq 0.001$.
[a]Percent was calculated by dividing the number that died by number of birds originally placed.

Table 2. Body weight

	Full-fed	Calorie-restricted
Body weight, kg	1.896a	1.423b
Caloric intake, average, kcal/bird/d	663	365

NOTE: Outcomes with different letters (a and b) were significantly different with $P \leq 0.001$.
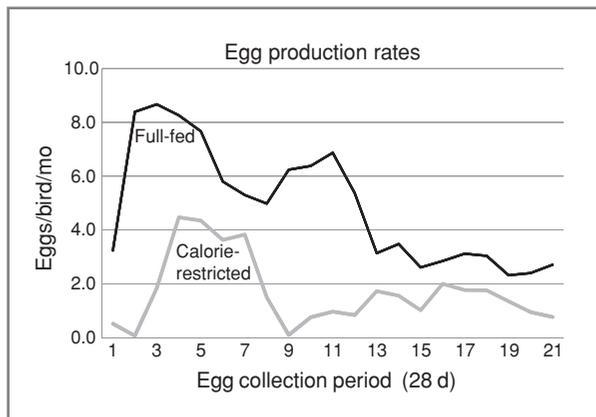

Figure 1. Ovulation rate.

Table 3. Cancer prevalence (based on histopathology)

	Full-fed	Calorie-restricted
Number of birds	171	253
OVAC	45	16
% Prevalence	26.3a	6.3b
OVDC	52	17
% Prevalence	30.4a	6.7b
Total carcinoma involving the reproductive tract[a]	57	26
% Prevalence	33.3a	10.3b

NOTE: Outcomes with different letters (a and b) across rows were significantly different with $P \leq 0.001$.
Abbreviation: OVDC, oviductal cancer.
[a]Often hens had lesions in both the ovary and oviduct so the total with reproductive adenocarcinomas is not the sum of ovarian and oviductal cancers.

71% of birds in the full-fed group were actively producing eggs at termination of the study, whereas only 29% of birds in the calorie-restricted group had fully functioning ovaries. Gross pathology results for birds that died prior to termination of the study, revealed 69 (26.4%) cancer suspects in the full-fed group compared with 5 (3.5%) cancer suspects in the calorie-restricted group.

Necropsy of birds remaining at the completion of the 2-year trial revealed that birds in the full-fed group had extensive abdominal fat involving the viscera and oviduct and a higher prevalence of cancers involving the reproductive tract, including cancers of the ovary and oviduct compared with birds that were calorie restricted (Table 3).

On the basis of histopathology, 45 (26.3%) birds in the full-fed group had ovarian adenocarcinoma compared with 16 (6.3%) birds in the calorie-restricted group. Cancers involving the oviduct were also more prevalent in the full-fed birds (52 birds or 30.4%) than in the calorie-restricted birds (17 birds or 6.7%). Tumor stages were similar irrespective of diet group (Table 4) with most tumors metastatic beyond the ovary. Overall adenocarcinomas involving the reproductive tract were more prevalent in full-fed birds (57 birds or 33.3%) than calorie-restricted birds (26 birds or 10.3%).

Discussion

Birds, like mammals, have a lower incidence of cancer and improved life expectancy when given a diet restricted in calories (35–45). Mortality rates were significantly higher in birds on the calorie-dense diet than for those fed a calorie-restricted diet. Histologic examination of tissues collected at the termination of the study clearly showed the prevalence of adenocarcinomas of the reproductive

Table 4. Cancer staging (ovarian adenocarcinoma), tumor numbers, and percentage of all tumors by tumor stage

Tumor stage	Full-fed (birds examined $n = 171$, tumor $n = 45$)	Calorie-restricted (birds examined $n = 253$, tumor $n = 16$)
1 (ovary only)	2 (4.4%)	3 (18.8%)
2 (ovary + oviduct)	7 (15.6%)	1 (6.3%)
3 (ovary ± oviduct with serosal metastasis)	10 (22.2%)	7 (43.8%)
4 (stage 3 + distant metastasis to liver, spleen, lung, kidney)	26 (57.8%)	5 (1.3%)

NOTE: Values are represented as number (percentage). Outcomes were not statistically significant with $P \leq 0.0711$.

tract was higher for birds fed a more calorie-dense diet than those on the calorie-restricted diet (33.3% vs. 10.3%, respectively). A weakness in our study is the lack of accurate classification of tumors in birds that died prior to the terminal necropsy. For these birds, gross pathology suggested a much higher rate of suspect cancers in the full-fed group than in the calorie-restricted group (26% vs. 3%, respectively). Although some tumor misclassification may have occurred, the trend toward greater tumor presence in birds that died in the full-fed group is unmistakable and is comparable to rates found by histopathology in the both groups of birds.

Egg production in both groups of hens was well below the normal 0.8 eggs per day. The hens were 2 to 4 years of age during this study, which is older that typical layers. In addition, the reproductive tracts of birds had become refractory to photostimulation due to intense lighting during the first 2 years of their lives. Even with lower egg production, records show a dramatic decrease in ovulation rate for the calorie-restricted group compared with the full-fed group. The full-fed group ovulated at a rate approximately 3 times that of the feed-restricted group. This reduction in ovulation rate correlates well with the reduction of adenocarcinoma in the ovary, which was reduced nearly 5-fold. The correlation between calorie restriction, decreased ovulation rate, and decreased ovarian adenocarcinoma demonstrated by this study is notable and analogous to that observed in women. The risk of OVAC in women is proportional to the number of lifetime ovulatory events. There is epidemiologic evidence that factors associated with fewer lifetime ovulations, such as pregnancy, lactation, or use of oral contraceptives, is associated with a marked decrease in OVAC risk. (2, 46–50). Although the mechanism responsible for this protective effect is not fully understood, the findings are supportive of the "incessant ovulation" hypothesis which states that the ovarian epithelium is subject to genetic damage during the wound and repair processes associated with ovulation (5).

The design of our study does not allow us to definitely conclude that the lower incidence of OVACs in the feed-restricted group was due to decreased ovulation, in that both caloric restriction and weight loss may beneficially impact OVAC risk and mortality. The reduction in the ovulation rate in the feed restriction group was associated with a 25% reduction in body weight. A positive association between excess body weight and mortality has been shown in both humans and animals. (35–39). Human mortality due to cardiovascular disease for example has long been associated with excess body weight (40). With regard to malignancy, there is a growing body of evidence that consistently shows an association between higher body weight and increased risk of cancers in various tissues (41–47). There is evidence that human death from OVAC is also influenced by body weight (38). Effects of body weight on tumor development and proliferation have been demonstrated in short-lived and long-lived mammalian species. Laboratory rodents and nonhuman primates have been used to demonstrate the effects of caloric restriction on life span and carcinogenesis. Moreschi and Rous observed that tumors transplanted into underfed mice did not grow as well as those transplanted into mice fed *ad libitum* (51, 52). In addition, free radicals generated by metabolism of some foods and environmental contaminants may promote establishment and proliferation of tumors (53, 54). Free radicals cause damage to DNA which, if not repaired, can facilitate carcinogenesis. Calorie restriction may be associated with a reduction in oxidative damage to DNA (53) as well as enhanced apoptosis. Apoptosis, which regulates cell death and is an important mechanism *in vivo* for elimination of genetically damaged cells, has been shown to be enhanced in animals fed a calorie-restricted diet (55). Apoptosis is important in the identification and self-destruction of cells which are old or damaged by toxins, ultraviolet light, or free radicals.

There are no previous studies that address the effects of caloric restriction on cancer prevalence and mortality in chickens. Although the mechanism of OVAC reduction in calorically restricted laying hens remains unknown, the dramatic difference in ovulation rates in this study suggests that ovulation or a combination of ovulation and other factors contributed to the reduction of adenocarcinoma in the calorie-restricted hens. Because calorie restriction directly affects ovulation rate in the chicken, it is unclear which factor has the greatest effect on reducing the risk of cancer development.

Disclosure of Potential Conflicts of Interest

Grant Support

References

1. Jemal J, Seigal R, Ward E, Murray T, Xu J, Smigal C. American Cancer Society statistics. CA: Cancer J Clin 2006;56:106–130.
2. Wu ML, Whitmore AS, Paffenbarger RS, Sarles DL, Kampert JB, Grosser S, et al. Personal and environmental characteristics related to epithelial ovarian cancer. Am J Epidemiol 1988;128:1216–27.
3. Green M, Clark J, Blayney D. The epidemiology of ovarian cancer. Semin Oncol 1984;11:209–26.
4. Whitmore A, Harris R, Itnry J. Characteristics related to ovarian cancer risks: collaborative analysis of twelve case-controlled studies. Am J Epidemiol 1992;136:1212–20.

5. Casagrande J, Pike M, Russ R, Louie E, Roy S, Henderson BE. Incessant ovulation and ovarian cancer. Lancet 1979;2:170–2.

6. Wilson JE. Adeno-carcinomata in hens kept in a constant environment. Poult Sci 1958;37:1253.

7. Fredrickson T. Ovarian tumors of the hen. Environ Health Perspect1987;73:35–51.

8. Lingeman C. Etiology of cancer of the human ovary: a review. J Natl Cancer Inst 1974;53:1603–18.

9. Barnes MN, Berry WD, Straughn JM, Kirby TO, Leath CA, Huh WK, et al. A pilot study of ovarian cancer chemoprevention using medroxyprogesterone acetate in an avian model of spontaneous ovarian carcinogenesis. Gynecol Oncol 2002;87:57–63.

10. Hamilton T, Young R, Louie K, Behrens BC, McKoy WM, Grotzinger KR, et al. Characterization of a xenograft model of human ovarian carcinoma which produces ascites and intraabdominal carcinomatosis in mice. Cancer Res 1984;44:5286–90.

11. Nishida T, Sugiyama T, Katabuchi H, Yakushiji M, Kato T. Histologic origin of rat ovarian cancer induced by direct application of 7,12-dimethylbenz(A)anthracene. Acta Obst Gynaec Jpn 1986;38:570–4.

12. Fu X, Hoffman R. Human ovarian carcinoma metastatic models constructed in nude mice by orthotopic transplantation of histologically-intact patient specimens. Anticancer Res 1993;13:283–6.

13. Testa J, Getts L, Salazar H, Liu Z, Handel LM, Godwin AK, et al. Spontaneous transformation of rat ovarian surface epithelial cells results in well to poorly differentiated tumors with a parallel range of cytogenetic complexity. Cancer Res 1994;54:2778–84.

14. Rose G, Tocco L, Granger G, DiSaia PJ, Hamilton TC, Santin AD, et al. Development and characterization of a clinically useful animal model of epithelial ovarian cancer in the Fischer 344 rat. Am J Obstet Gynecol 1996;175:593–9.

15. Silva E, Tornos C, Fritsche H, el-Naggar A, Gray K, Ordonez NG, et al. The induction of benign epithelial neoplasms of the ovaries of guinea pigs by testosterone stimulation: a potential animal model. Mod Pathol 1997;10:879–883.

16. Yoshida Y, Kamitani N, Sasaki H, Kusumi K, Tominaga T, Kotsuhi F. Establishment of a liver metastatic model of human ovarian cancer. Anticancer Res 1998;18:327–32.

17. Silva E, Tomos C, Deavers M, Kaisman K, Gray K, Gershenson D. Induction of epithelial neoplasms in the ovaries of guinea pigs by estrogenic stimulation. Gynecol Oncol 1998;71:240–6.

18. Kiguchi K, Kubota T, Aoki D, Udagawa Y, Yamanouchi S, Saga M, et al. A patient-like orthotopic implantation nude mouse model of highly metastatic human ovarian cancer. Clin Exp Metastasis 1998;16:751–6.

19. Xu Y, Silver D, Yang N, Oflazoglu E, Hempling RE, Piver MS, et al. Characterization of human ovarian carcinomas in a SCID mouse model. Gynecol Oncol 1999;72;161–70.

20. Boyd J. Mouse models of gynecologic pathology. N Engl J Med 2005;352:2240–2.

21. Connolly D, Bao R, Nikitin A, Stephens KC, Poole TW, Hua X, et al. Female mice chimeric for expression of the simian virus 40 Tag under control of the MISIIR promoter develop epithelial ovarian cancer. Cancer Res 2003;63:1389–97.

22. Flesken-Nikitin A, Choi K, Eng J, Shmidt E, Nikitin A. Induction of carcinogenesis by concurrent inactivation of p53 and Rb1 in the mouse ovarian surface epithelium. Cancer Res 2003;63:3459–63.

23. Dinulescu D, Ince T, Quade B, Shafer S, Crowley D, Jacks T. Role of K-ras and Pten in the development of mouse models of endometriosis and endometrioid ovarian cancer. Nat Med 2005;11:63–70.

24. Liu J, Yang G, Thompson-Lanza A, Glassman A, Hayes K, Patterson A, et al. A genetically defined model for human ovarian cancer. Cancer Res 2004;64:1655–63.

25. Sallinen H, Anttila M, Narvainen J, Ordén MR, Ropponen K, Kosma VM, et al. A highly reproducible xenograft model for human ovarian carcinoma and application of MRI and ultrasound in longitudinal follow-up. Gynecol Oncol 2006;103:315–20.

26. Crist K, Zhang Z, You M, Gunning WT, Conran PB, Steele VE, et al. Characterization of rat ovarian adenocarcinomas developed in response to direct instillation 7,12-dimethylbenz[a]anthracene (DMBA) coated suture. Carcinogenesis 2005;26:951–7.

27. Shaw T, Senterman M, Dawson K, Crane C, Vanderhyden B. Characterization of intraperitoneal, orthotopic, and metastatic xenograft models of human ovarian cancer. Mol Ther 2004;10:1032–42.

28. Stewart S, Querec T, Ochman A, Gruver BN, Bao R, Babb JS, et al. Characterization of a carcinogenesis rat model of ovarian preneoplasia and neoplasia. Cancer Res 2004;64:8177–83.

29. Vanderhyden B, Shaw T, Ethier J. Animal models of ovarian cancer. Reprod Biol Endocrinol 2003;1:67.

30. Stakleff K, Von Gruenigen V. Rodent models of ovarian cancer research. Int J Gynecol Cancer 2003;13:405–12.

31. Repasky E, Tims E, Prichard M, Burd R. Characterization of mild whole body hyperthermia protocols using human breast, ovarian, and colon tumors in severe combined immunodeficient mice. Infect Dis Obstet Gynecol 1999;7:91–7.

32. Kiguchi K, Kubota T, Aoki D, Udagawa Y, Yamanouchi S, Saga M. A patient like orthotopic implantation nude mouse model of highly metastatic human ovarian cancer. Clin Exp Metastasis 1998;16:751–6.

33. Orsulic S, Li Y, Soslow R, Vitalae-Cross L, Gutkind J, Varmus H. Induction of ovarian cancer by defined multiple genetic changes in a mouse model system. Cancer Cell 2002;1:53–62

34. Hakim A, Barry C, Barnes J, Anderson KE, Petitte J, Whitaker R, et al. Ovarian adenocarcinomas in the laying hen & women share similar alterations in p53, ras, & HER-2/neu. Cancer Prev Res 2009; 2:114–21.

35. Manson J, Willett W, Stampfer M, Colditz G, Hunter D, Hankinson S, et al. Body weight and mortality among women. N Engl J Med 1995;333:677–85.

36. Stevens J, Plankey M, Williamson D, Thun MJ, Rust PF, Palesch Y, et al. The body-mass index-mortality relationship in white and African-American women. Obes Res 1997;6:268–77.

37. Linsted K, Singh P. Body mass and 26 y risk of mortality among men who never smoked: a re-analysis of men from the Adventist Mortality Study. In J Obes Res Relat Metab Disord 1998;22:544–58.

38. Calle E, Thun M, Petrelli J, Rodriquez C, Heath C Jr. Body-mass index and mortality in a prospective cohort of U.S. adults. N Engl J Med 1999;341:1097–1105.

39. Kopelman P. Obesity as a medical problem. Nature 2000;404:635–643.

40. Willett W, Manson J, Stampfer M, Colditz GA, Rosner B, Speizer FE, et al. Weight, weight change, and coronary heart disease in women: risk within the normal weight range. JAMA 1995;273:461–5.

41. World Cancer Research Fund. Food, Nutrition and the Prevention of Cancer: A Global Perspective. Washington, DC: American Institute for Cancer Research; 1997. p. 371–3.

42. Carroll K. Obesity as a risk factor for certain types of cancer. Lipids 1998;33:1055–59.

43. Bergstrom A, Pisani P, Tenet V, Wolk A, Adami H-O. Overweight as an avoidable cause of cancer in Europe. Int J Cancer 2001;91:421–30. Erratum in: Int J Cancer 2001;92:927.

44. IARC Handbooks of Cancer Prevention. Vol. 6. Weight Control and Physical Activity. Lyons, France: International Agency for Research on Cancer; 2002.

45. Peto J. Cancer epidemiology in the last century and the next decade. Nature 2001;411:390–5.

46. Franceseschi S, Parazzini F, Negri E, Booth M, La Vecchia C, Beral V, et al. Pooled analysis of three European case-control studies of epithelial ovarian cancer: III. Oral contraceptive use. Int J Cancer 1991;49:61–5.

47. Gross T, Schlesselman J. The estimated affect of oral contraceptive use on the cumulative risk of epithelial ovarian cancer. Obstet Gynecol 1994;83:419–24.

48. Lee N, Wingo P, Gwynn M, Rubin G, Kendrick J, Webster L, et al. The reduction in risk of ovarian cancer associated with oral contraceptive use. N Engl J Med 1987;316:650–5.

49. Rosenberg L, Palmer J, Zauber A, Warshauer ME, Lewis JL Jr, Strom BL, et al. A case-control study of oral contraceptive use and invasive epithelial ovarian cancer. Am J Epidemiol 1994;139:654–61.

50. Stanford J, Thomas D, Ray R, Noonan E. Epithelial ovarian cancer and combined oral contraceptives: the WHO collaborative study of neoplasia and steroid contraceptives. Int J Epidemiol 1989;18:538–45.

51. Moreschi C. Beziehungen zwischen Ernahrung und Tumorwachstum. Zeitschrift fur Immunitatsforsch 1909;2:661–75.

52. Rous P. The influence of diet on transplanted and spontaneous tumors. J Exp Med 1914;20:433–51.

53. Lgun A, Akman S, Serdar M, Kutluay T. Oxidative phosphorylation enzyme complexes in caloric restriction. Exp Gerontolog 2002;37:639–45.

54. Kritchevsky D. Caloric restriction and cancer. J Nutr Sci Vitaminol 2001;47:13–19.

55. Mukherjee P, El-Abbadi M, Kasperzyk J, Ranes M, Seyfried T. Dietary restriction reduces angiogenesis and growth in an orthotopic mouse brain tumour model. Brit J Cancer 2002;86: 1615–21.

Cancer Prevention Research

Reduction of Ovarian and Oviductal Cancers in Calorie-Restricted Laying Chickens

Donna K. Carver, H. John Barnes, Kenneth E. Anderson, et al.

Updated version	Access the most recent version of this article at: doi:10.1158/1940-6207.CAPR-10-0294
Supplementary Material	Access the most recent supplemental material at: http://cancerpreventionresearch.aacrjournals.org/content/suppl/2013/04/16/1940-6207.CAPR-10-0294.DC1

Cited articles	This article cites 52 articles, 8 of which you can access for free at: http://cancerpreventionresearch.aacrjournals.org/content/4/4/562.full#ref-list-1
Citing articles	This article has been cited by 2 HighWire-hosted articles. Access the articles at: http://cancerpreventionresearch.aacrjournals.org/content/4/4/562.full#related-urls

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://cancerpreventionresearch.aacrjournals.org/content/4/4/562. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.